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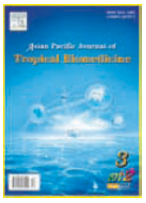

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In vitro and invivo evaluation of hepato protection and anti ulcer activities of piperine gastro retentive micropspheres

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ABSTRACT

Objective: In the recent years of research, the interest on herbal medicine is continuously increasing. Piperine is an alkaloid extracted from ripen fruits of *Piper nigram* and was proved in literature for its hepato protective and anti ulcer properties majorly through anti oxidant capability. **Methods:** In the present investigation gastro retentive, both floating and mucoadhessive microspheres are evaluated for the hepato protection in paracetamol induced model and gastric protection in rats and a comparison was done with conventional microspheres and pure form of piperine. **Results:** The results clearly showed the significant decrease in the serum levels of the marker enzymes in hepato protective study supported by histopathology along with reduced ulcer index in anti ulcer activity. **Conclusion:** This clearly indicates that there is an increase in both activities of floating microspheres, mucoadhessive microspheres when compared with the PP and conventional microspheres.

1. Introduction

Liver is the most important organ of human body and it plays an important role detoxification and also the first victim to toxins leading to hepatotoxicity [1]. Most of the liver damages are induced by lipid peroxidation and other oxidative damages caused by toxins. Exogenous factors like smoke, alcohol, stress, fatty food will trigger free radical generation which further leads to mucosal ischemia, excess secretion of hydrochloric acid, pepsin ultimately resulting in ulcers [2]. In India, more than 93 medicinal palnts are used in different combination in the preparations of 40 patended herbal formulations [3, 4]. From the herbal source various plants with antioxidant capability as major mechanism along with other mechanisms are used for the hepato and gastric protection [5].

Black pepper is a common spice of intercontinental food and is widely used as carminative, stimulant and also for the treatment of rheumatism, diarrhea, dysentery, cholera and menstrual pain. It is also used in folk medicine for stomach disorders and digestion problems. Piperine from *Piper nigram* was proved in literature for its hepato protection by reducing the lipid peroxidation [6-8]. Being anti oxidant, black pepper appears to protect gastric mucosa by the stimulation of bioenergetic process and endogenous levels of co enzyme Q10. The presence of spasmodic (cholinergic) and anti spasmodic (opiod agonist and calcium antogonist) effects of piperine give further support its use in gastro intestinal disorders [9, 10].

From the recent scientific and patent literature, it is evident that there is clear inclination towards the gastroretentive multi unit dosage forms. In gastroretentive dosage forms, while the system retains in gastric environment, the drug is released slowly at desired rate. Thus these are advantageous because of their ability to control the release of the drug at the gastric site without getting cleared from the tract [11].

In our present research, the main objective is to develop gastroretentive floating and mucoadhessive microspheres of piperine and to evaluate their invivo hepato and gastro protection in comparison with conventional microspheres and pure piperine.

2. Materials And Methods

2.1. Materials

Piperine (98%) from Alfa Aesar, UK and HPMC (Hydroxy Propyl Methyl Cellulose), Carbopal were purchased from SD fine Chemicals Mumbai. Commercially available assay kits for the estimation of serum enzymes were purchased and all other chemicals were of analytical grade.

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2.2. Methods

2.2.1. Preparation of microspheres by Emulsification solvent evaporation method [12]

Piperine microspheres were prepared by using solvent evaporation method. In brief the procedure includes, Piperine along with polymers were dissolved in acetone. This mixture was then emulsified in light liquid paraffin containing 3% span 80 with continuous stirring at 950rpm at room temperature for 4.5 hours. After evaporation of the acetone the formed microspheres were filtered and washed with petroleum ether to remove the traces of light liquid paraffin. The same method was followed for the preparation of floating microspheres, mucoadhessive microspheres and Conventional microspheres. In floating microspheres, ethyl cellulose, hydroxy propyl methyl cellulose and calcium carbonate were usedas polymers. In mucoadhessive microspheres ethyl cellulose, hydroxy propyl methyl cellulose and carbopal were used. In Conventional microspheres, only ethyl cellulose was used. The prepared microspheres were evaluated [13- 15] for encapsulation efficiency, particle size, % drug release and buoyancy for floating microspheres, mucoadhesion for mucoadhessive microspheres.

2.2.2. In Vivo Hepatoprotective Activity [16]

Adult albino wistar male rats weighing 150-200g were taken from the Technocrats institute of Technology, Bhopal. The animals were maintained in well ventilated room with natural12 hours day-night cycle and at room temperature in propylene cages. Animals were maintained with standard diet, food and water ad libitum. The protocol was approved by Animal Ethics constituted as per CPCSEA Guidelines (Ref No. TIT/IAEC/831/P′col/2010/1). Paracetamol induced hepatotoxicity in rats was used as a model to determine the hepatoprotective activity. The rats were divided into seven groups with six animals in each group and were given dose schedule as

Control: Animals were not given with either paracetamol or treatment for 14 days.

Group 1: Animals were given with paracetamol 2mg/kg on 6th day per oral and this served as toxic control.

Group 2: Animals were treated with 2 mg/kg, p.o of Slymarin for 1-6 days and on 6th day animals were given with paracetamol 2mg/kg p.o.

Group 3: Animals were treated with 300 mg/kg, p.o of Piperine for 1-6 days and on 6th day animals were given with paracetamol 2mg/kg p.o.

Group 4: Animals were treated with 300 mg/kg, p.o of Piperine floating formulation for 1-6 days and on 6th day animals were given with paracetamol 2mg/kg p.o.

Group 5: Animals were treated with 300 mg/kg, p.o of Piperine mucoadhessive formulation for 1-6 days and on 6th day animals were given with paracetamol 2mg/kg p.o.

Group 6: Animals were treated with 300 mg/kg, p.o of Piperine ethyl cellulose microspheres for 1-6 days and on 6th day animals were given with paracetamol 2mg/kg p.o.

On the 7th day the animals were sacrificed and various parameters were analyzed. At the end of the experimental period animals were sacrificed by cervical decapitation under mild pentobarbitone anesthesia, blood was collected and the serum was separated by centrifuging at 3,000 rpm for 10 min. The collected serum was used for the assay of marker enzymes. The enzymes like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bile content and total protein content were determined. After draining the blood, liver samples were excised, washed with normal saline and processed separately, for histological observations. The liver was imucoadhessive microsphereediately removed and fixed in formalin, serially sectioned and microscopically examined after staining with hematoxylin and eosin to analyse pathological changes.

2.2.3. Invivo anti ulcer activity [17]

Adult albino wistar male rats weighing 150-200g were taken from the Technocrats institute of Technology, Bhopal. The animals were maintained in well ventilated room with natural12 hours day-night cycle and at room temperature in propylene cages. Animals were maintained with standard diet, food and water ad libitum. The protocol was approved by Animal Ethics constituted as per CPCSEA Guidelines (Ref No. TIT/IAEC/831/P′ceutics/2010/5).

The dose, 300 mg/kg was selected for the conduct of the experiments were based on preliminary experiments conducted on the pharmacological activity of Black pepper. The route of administration of the aqueous (water) suspension was oral (gastric intubation) in all the experiments. The animals in the test groups were orally administered 1 ml per rat of necrotizing agent (80% ethanol) which is known to produce gastric lesions.

The rats were divided into six groups with six animals in each group and were given dose schedule as

Group I: Animals were not given with either ethanol or treatment for 14 days. This group served as control;

Group II: Animals were given with ranitidine 100mg/kg per oral and this served as standard control.

Group III: Animals were treated with 300 mg/kg, p.o of Piperine

Group IV: Animals were treated with 300 mg/kg, p.o of Piperine floating formulation

Group V: Animals were treated with 300 mg/kg, p.o of Piperine mucoadhessive formulation

Group VI: Animals were treated with 300 mg/kg, p.o of Piperine ethyl cellulose microspheres

Based on the gastric emptying in fasted rats, formulations were given 30 min before the necrotizing agent. Animals were sacrificed under ether anesthesia 1 hr after treatment with ulcerogenic agent. The stomach was excised and opened along the greater curvature. After washing with normal saline, the gastric lesions were quantified using a magnifier. If there is no ulceration, hyperemia, hemorrhagic spots, 1-5 small ulcers, many small ulcers, many large ulcers and stomach full of ulcers then the ulcer index was given as 0, 0.5, 1, 2, 3, 4, 5 and 6 respectively.

2.2.4. Statistical analysis

The data was expressed as mean±SD. Data were analyzed by Dunnet′s analysis of variance (ANOVA) to compare all groups against control. Results were considered statistically significant at $P < 0.001$ and $P<0.005$.

3. Results

Microspheres were prepared by solvent evaporation method and the evaluation parameters were given in the figure 1.